SEM studies on immature stages of weaver ant *Oecophylla smaragdina* (Fabricius, 1775) (Hymenoptera: Formicidae) from India

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Abstract

The present study has been designed to focus on scanning electron microscopy of immature stages of weaver ant *Oecophylla smaragdina* and this work would provide an insight to employ larval diagnostic features as a supplement to remove confusion in very closely related species with better resolution and more precision. Presence of a prepupal stage in *O. smaragdina* has been indicated by present study, which was not reported earlier (Fig. 4).

Keywords: *SEM, Immature stages, Oecophylla smaragdina, India.*

Introduction

Ants are social insects which belong to family Formicidae, and along with wasps and bees, they constitute order Hymenoptera, one of the advanced orders. Evidence for the success of ants is provided by the estimates of their total biomass which is nearly 15% of the entire terrestrial animal biomass. The species under consideration *Oecophylla smaragdina* (Fabricius, 1775) distributed in India, Australia and South-east Asia is in use as effective biological control agent. Immature stages of this species are also used as food supplement in various regions of South–east Asia. As the larvae are important for the well being of the colony, the physiology and behavior of the colony with reference to the larvae must be learnt. Wheeler and Wheeler (1953, 1960a, 1960b, 1964a, 1964b, 1964c, 1966, 1970, 1976, 1980, 1982, 1986a, 1986b, 1986c, 1986d, 1989a, 1989b, 1989c and 1991) described the larvae of over 700 ant species and explained morphology of the generalized ant larva using simple optical microscope. They emphasized the importance of larval descriptions to myrmecology and concluded that certain larval characters can be applied to species level taxonomy and systematics in various ant genera

As their study included a single mature larva and moreover, the work was based on optical microscopy, hence they were not able to elucidate minute details. More recently, few workers (e.g. Fox *et al.*, 2007) paid more attention to minute larval structures and carried out scanning electron microscopy. But the case of all important weaver ant *Oecophylla* could not get recognition. With this back drop, the present study was designed to focus on scanning electron microscopy of immature stages of weaver ant *Oecophylla smaragdina*. It is hoped that this work would provide an insight to employ larval diagnostic features as a supplement to remove confusion in very closely related species with better resolution and more precision.

Materials and Methods

1. Immature stages of *Oecophylla smaragdina* were collected from mango, citrus and guava trees in Horticulture Department, Punjabi University, Patiala and also from surrounding areas (Ropar, Hoshiarpur and Anandpur Sahib).

2. The larvae were fixed in Dietrich's Solution for 24 hours.

3. After fixing in Dietrich Solution these were preserved in 80% alcohol.

4. Then the larvae were separated into three instars according to their maximum head capsule width.

5. Measurements were made with a compound microscope equipped with an ocular micrometer.

6. Body lengths from 50 larvae from each instar were measured for more accuracy.

7. After separation and measurements, larvae (N=10) from each instar were prepared for Scanning Electron

microscopy analysis with following protocol;
(i) Samples were post-fixed in 1% Osmium Tetraoxide.
(ii) These were dehydrated in graded acetone series.
(iii)Then the specimens were vacuum dried in desiccator.
(iv) Specimens adhered to the double-face adhesive carbon tape were coated with gold in a gold ion sputter coater (HITACHI E-1010).
(v) Then the samples were analyzed under a HITACHI S -3400 N Scanning Microscope.

8. The terminology used for the description of the larvae of *Oecophylla smaragdina* follows Wheeler and Wheeler (1976) and Fox *et al.* (2007). Body hairs were measured at full length and body length is presented both in straight length (N=50 for each instar) and length through spiracles, which was taken from only one larva of each instar still in good body shape after being mounted on glass slide.

Pupae lengths were measured on a straight line from the top of the head capsule to the tip of the abdomen. Concerning other measurements (of head capsule, mouthparts, hairs, etc.) only of one individual per instar are presented in the description. All body measurements are presented as mean ± standard error.

Results and Discussion

The larvae of *O. smaragdina* have been previously described by Wheeler and Wheeler (1976, 1986b), although without the knowledge of the number of larval instars and that too based on five specimens only.

According to Wheeler and Wheeler (1976) a generalized ant larva possesses the following features: "an ant larva is soft, whitish, legless grub with a distinct, soft and hypognathous head on the anterior end of body, followed by 13 distinct somites posteriorly. Body profile is pogonomyrmecoid i.e. diameter greater near the middle of the abdomen, decreasing gradually towards the posterior end, which is rounded. Thorax is more slender than abdomen and form a ventrally curved neck. Leg vestiges are present as pairs of short transverse lines near the posterior borders of each thoracic somite. Gonopod vestiges are present as a pair of short transverse lines on the ventral surface of one or more abdominal somites VII, VIII and IX. Anus is a transverse slit on the abdominal somite X, slightly ventral to the most posterior point on the somite. Ten pairs of minute and uniform spiracles, a pair each on mesothorax, metathorax and abdominal somite I to VIII are present. Integument of body is spinulose and body is furnished with unbranched hairs. Cranium is subhexagonal in anterior view. Integument of the cranium is smooth with a few sensilla. Antennae bear three sensilla, each of which bear a minute spinule. Clypeus is marked off by grooves. Mandibles, palps, galea and pleurostoma are sclerotized. Labrum is bilobed thick flap. Maxillae are with the conoidal lacinia. Palps are paxilliform each with 5 sensilla. Labrum is hemispheroidal. Hypopharynx is densely spinulose".

In tribe Oecophyllini the body profile is Oecophylloid type. Very few, minute, smooth, unbranched and acute body hairs are present. Antennae are minute. Head hairs are few in number, very short and spike-like. Labrum is small and bilobed. Only two hair are present on anterior surface. Chiloscleres are lacking. Mandibles are very small and dolichoderoid. Maxillae are broad and apparently adnate. Palps and galea are very small. In the present SEM studies on *Oecophylla smaragdina* the number of larval instars were established by measuring maximum head capsule widths of the larvae (Solis, 2007) and it has been observed that the number of larval instars for *Oecophylla smaragdina* is three, which are discussed in detail as follows:

First larval instar

Body- The first larval instars of *Oecophylla smaragdina* are whitish in colour. Wheeler and Wheeler (1976) has divided the body profiles into 12 types namely Pogonomyrmecoid, Pheidoloid, Dolichoderoid, Attoid, Myrmecoid, Crematogastroid, Aphaenogasteroid, Platytheroid, Leptanilloid, Leptomyrmecoid, Oecophylloid and Rhopalomastigoid. Body profile of *O. smaragdina* is oecophylloid i.e. body is plump, sausage-shaped and slightly curved. Diameter of the body is nearly uniform. No neck is formed (Fig. 1a and 1b). Head is on the anterior end. Anus is slightly sub-terminal in position with no differentiation of lips perhaps due to the preservation of the specimens. Head capsule is proportionally small in relation to the body size (Fig.1a). There are only few, unbranched, sparsely distributed body hairs measuring approximately 5.27µm in length (Fig. 1c). Hairs are abundantly distributed near the anterior surface of thorax measuring 57.34µm in length (Fig. 1d). Body protuberances are divided into three groups by Wheeler and Wheeler (1976); the leptaenilline protuberances; welts which are low, elongate and narrow protuberances and tubercles include all other shapes like bosses if they are low, convex and subcircular. Body protuberances in case of *O. smaragdina* are in the form of spike like structures measuring 18.3µm in length and boss which is an elevated structure with a rounded

terminus measuring 18.3µm (Fig.1e). The integument of the whole body is densely covered with small, about 5.98µm long spike like protuberances (Fig. 1f). Ten pairs of spiracles, a pair each on the mesothorax, metathorax and eight anterior abdominal somites are present with the diameter of 1.81µm (Fig. 1b and 1g). Length of a straight stiff larva is not comparable to the length of the curved flexible larva. So, two lengths are measured on straight larva *viz.*, straight length and length through spiracles. Straight length is measured from the dorsum of the prothorax to the posterior end of the body and length through spiracles from the front of the head through all the spiracles to the anus. Straight body length is 951.5µm; range 883-1020µm and length through spiracles is 1514µm.

Head Capsule- Cranium 306µm high X 340µm wide and is roughly sub heptagonal. A single cephalic seta present, measuring 36.6µm in length on the tip of the cranium (Fig.1h). Antennae are observed as slight elevations from the head measuring 5.3µm in width, with three sensilla on them (Fig. 1i). Head surface is smooth with simple hairs measuring 19.4µm in length on it. Very few, short and spike like hairs observed. Distribution of hairs is not symmetrical.

Mouthparts- Clypeus not clearly delimited from the cranium, upper surface of clypeus smooth, without sensilla. A distinct row of simple hairs with 42.06µm in length is present lining the distal clypeal border. Labrum small, measuring 62.1µm in length, bilobed and with only two simple hairs on the anterior surface (Fig. 1j).

Second Larval Instar

Body-Body profile is Oecophylloid type as observed in the first instar, but head is slightly terminal in position, neck becomes more curved, body becomes cylindrical and narrow (Fig. 2a). Anus is clearly sub-terminal in position measuring 22.8µm in transverse length (Fig. 2b). Body hairs are minute, smooth, unbranched and acute measuring in 46.6µm in length. Integument of the body is covered with small spike like protuberances (Fig. 2c and 2d). Ten pairs of spiracles are present with 2.41µm diameter (Fig. 2e). Body length is 1815µm; range 1700-1930µm and length through spiracles is 1821.5µm.

Head Capsule- Cranium 332µm high X 433µm wide (Fig. 2f). Antennae are distinct, 12.0µm in diameter (Fig. 2g). Head hairs are smooth, simple straight, curved and measure about 47.6µm in length (Fig. 2f and 2h).

Mouthparts- Labrum bilobed, 88.2µm wide with two simple hairs on its anterior surface (Fig. 2h and 2i). Mandibles are small, simple, sharp-pointed, 39.4µm long and 9.2µm wide at the base and dolichoderoid in shape without any medial teeth (Fig. 2i, and 2j). Maxillae are broad, adnate type and measure 25.7µm in length and 20.8µm in width at the base. Maxillary palpus 10.65µm long. Galea is simple 6.68µm long (Fig. 2i and 2j). Labium is 88.2µm wide (Fig. 2h and 2i).

Third Larval Instar

Body- Body profile is as in the second instar (Fig. 3a). Anus is sub-terminal in position (Fig. 3b). Body hairs very sparsely distributed, about 24.02µm long. Spike like body protuberances measuring 3.43µm in length are present on the whole body surface (Fig. 3c). Spiracles are 5.93µm in diameter (Fig. 3d). Body length is 2320µm; range 2070-2570µm. Length through spiracles is 3010µm.

Head Capsule- Cranium 431µm high X 495µm wide. All head hairs are simple, unbranched, acute and measure about 53.06µm in length (Fig. 3e and 3h). Cephalic seta is 43.7µm long and 31.8µm wide (Fig. 3e). Antennae are 14.2µm wide with three sensilla (Fig. 3f).

Mouthparts- Clypeus is not clearly delimited from the cranium. Labrum 125µm wide with two hairs, which are 13.7µm long on its anterior surface (Fig. 3g and 3h). Mandibles are small, dolichoderoid in shape, 23.9µm long (Fig. 3g and 3h).

To sum up, the description of the larva confirms the general morphological aspects originally observed by Wheeler and Wheeler (1976) with some marked differences. It has been observed during the present study that the profile of the larva remains Oecophylloid in all the three instars and size of body increases as the larva grows (Fig. 1a, 2a and 3a). Straight body length of the first instar was 951.5µm which increases upto 1815µm in second instar and 2320µm in third instar. Length through spiracles also increases. Position of anus shifts from slightly ventral in first instar to subterminal in second instar which remains the same in the third instar (Fig. 2b and 3b). Wheeler and Wheeler (1976) observed the body protuberances in the form of welts and bosses, but small spike like protuberances on the whole body surface of all the three instars has been observed which are already known to be useful to the larva in following manners (Fig. 1f, 2c, 2d and 3c):

1. They provide support to the body by creating an airspace between body and substrate.
2. Some of the hairy tubercles afford protection against cannibalism.
3. They help larvae in the process of attachment.
4. Tubercles act as exudate organs which secrete onto their surfaces substances to which workers are very fond, thus help in trophallaxis.
5. They help in holding food.

Variation in hair distribution on the body between

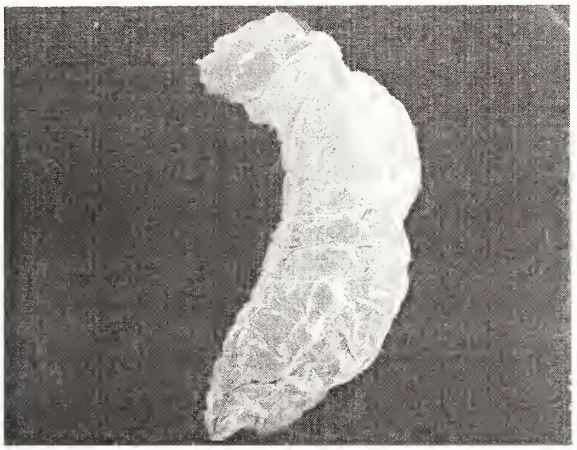

Fig.1a

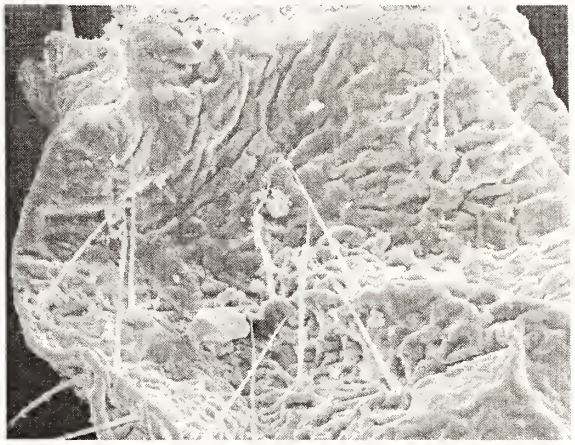

Fig.1d

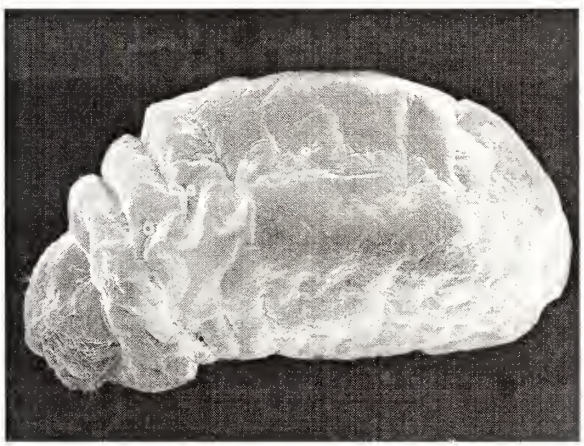

Fig.1b

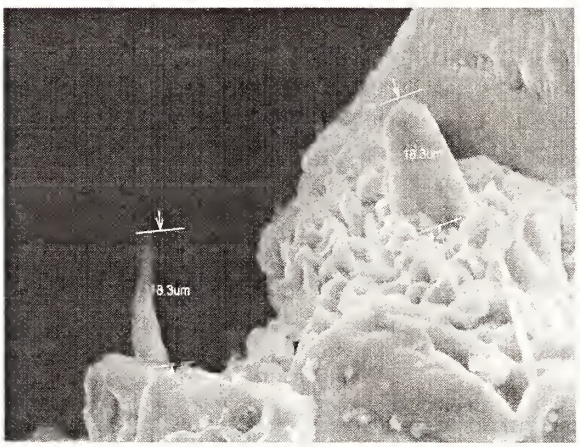

Fig.1e

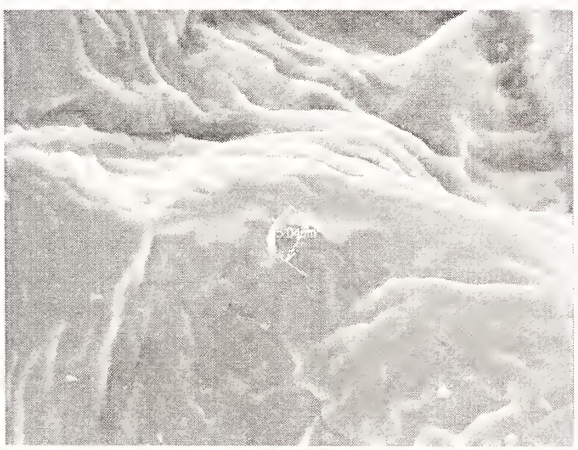

Fig.1c

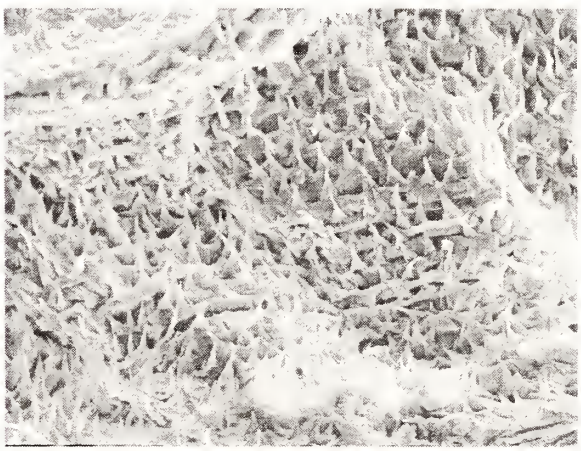

Fig.1f

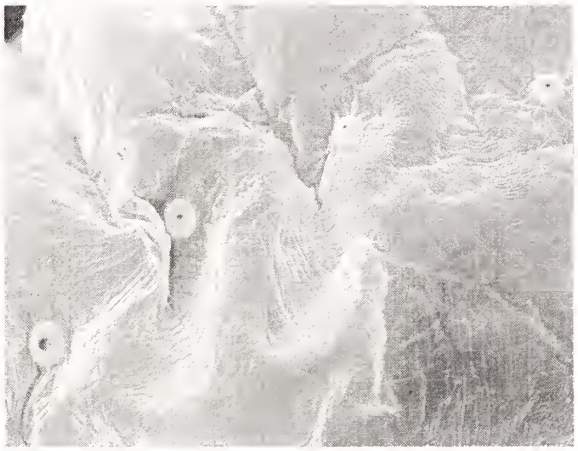

Fig.1g

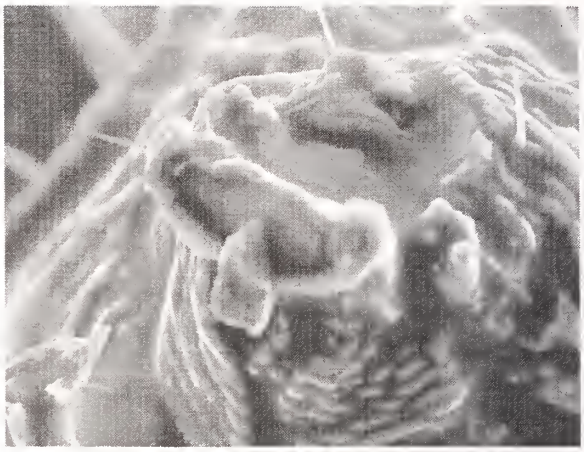

Fig.1j

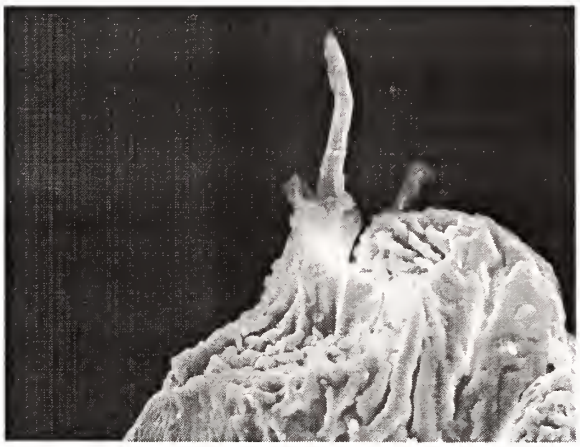

Fig.1h

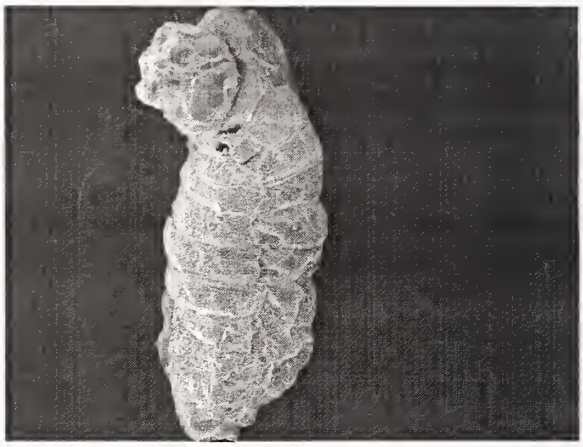

Fig.2a

Fig.1i

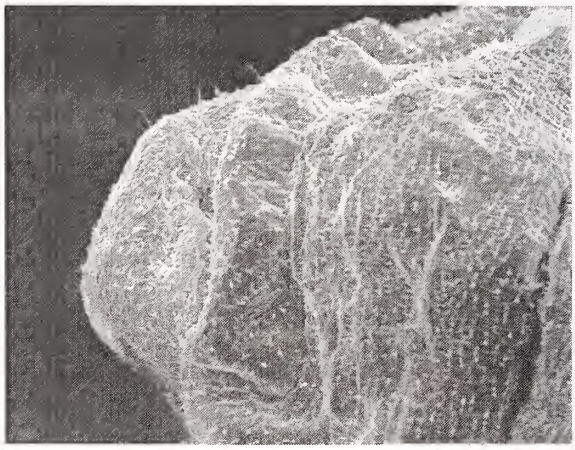

Fig.2b

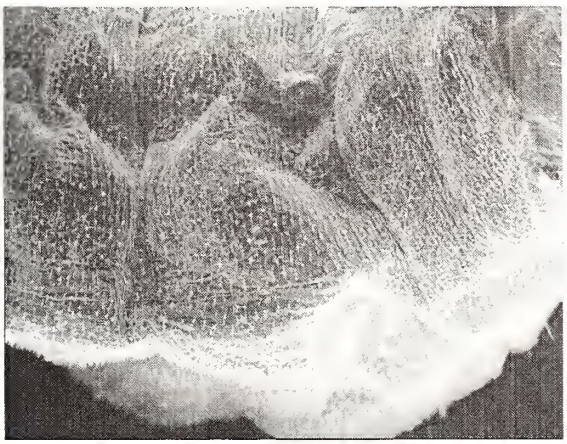

Fig.2c

Fig.2f

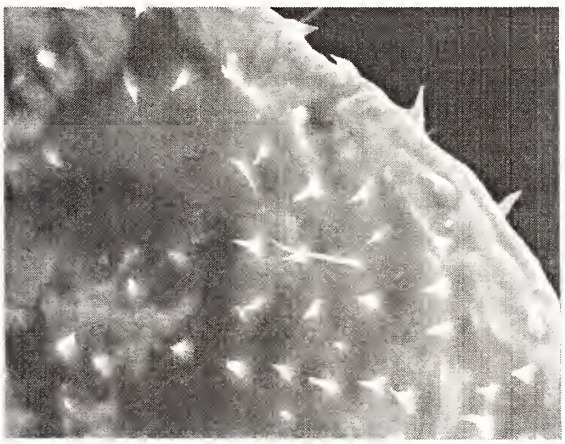

Fig.2d

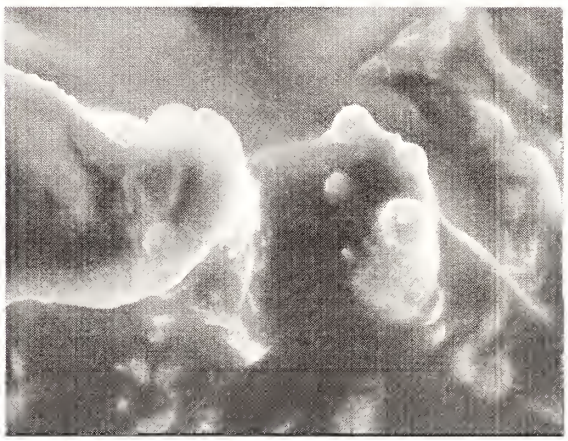

Fig.2g

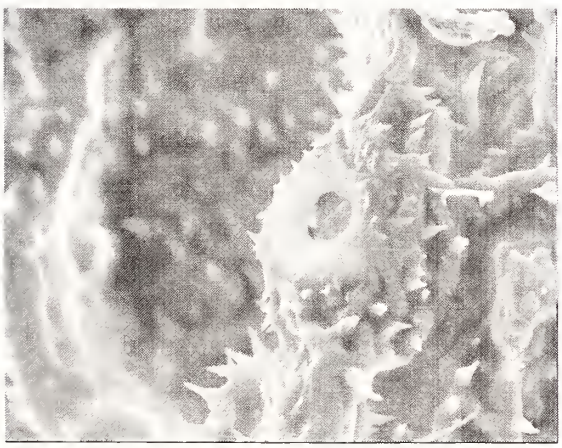

Fig.2e

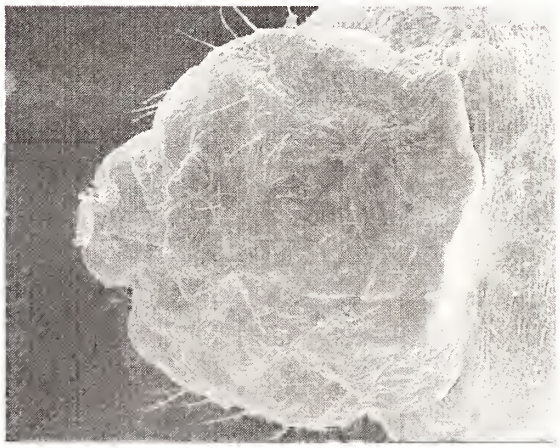

Fig.2h

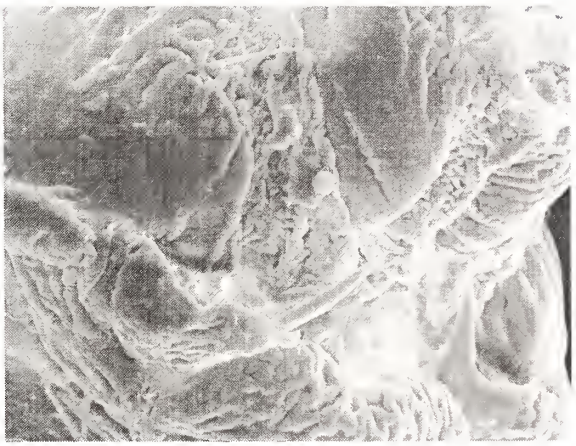

Fig.2i

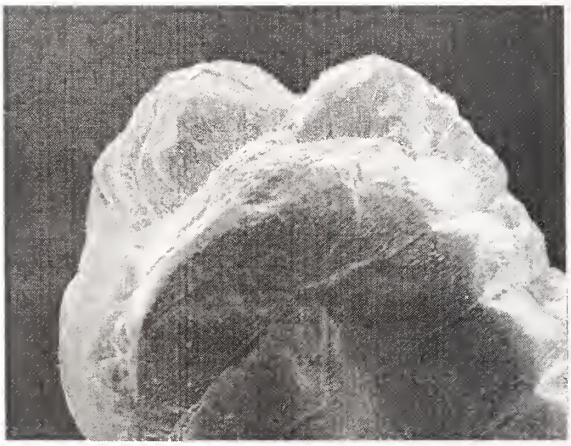

Fig.3b

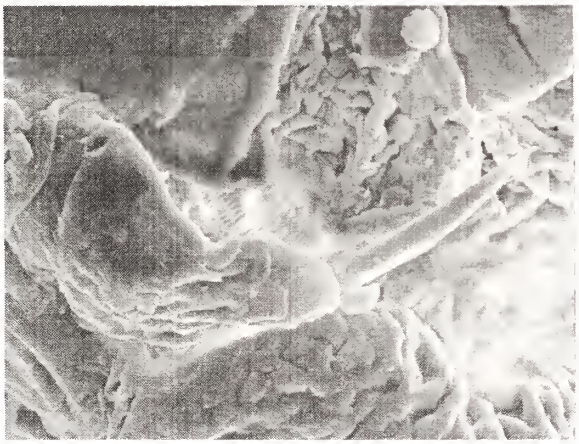

Fig.2j

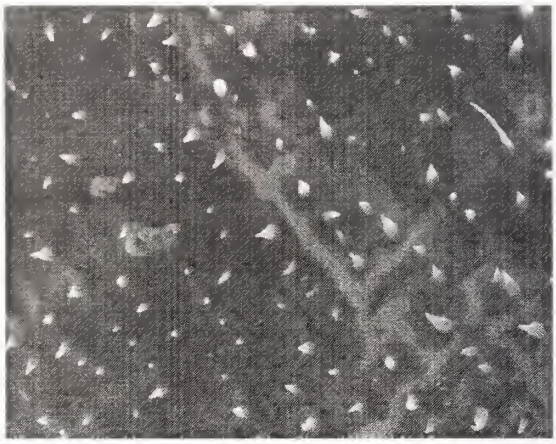

Fig.3c

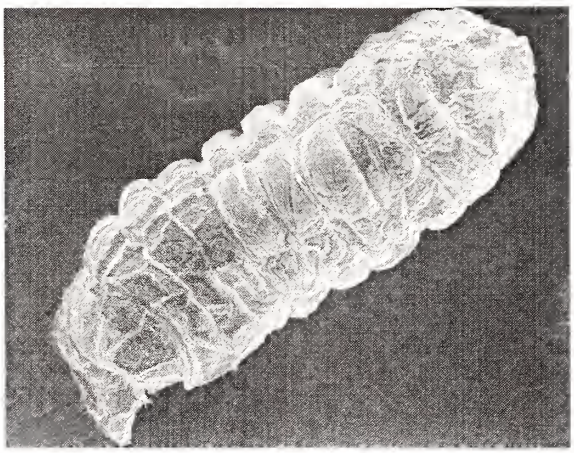

Fig.3a

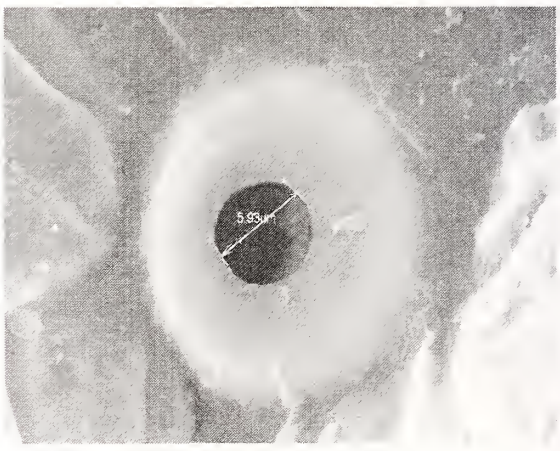

Fig.3d

Fig.3e

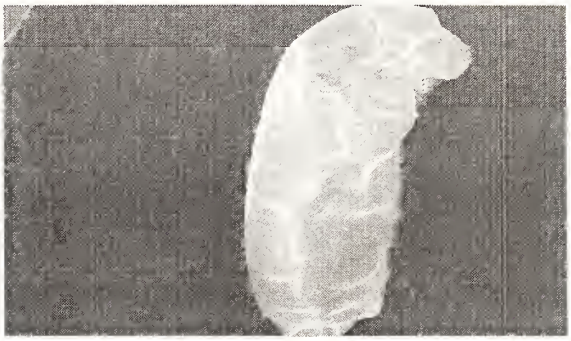

Fig.4

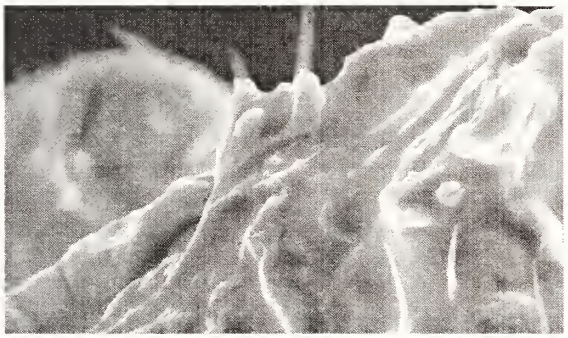

Fig.3f

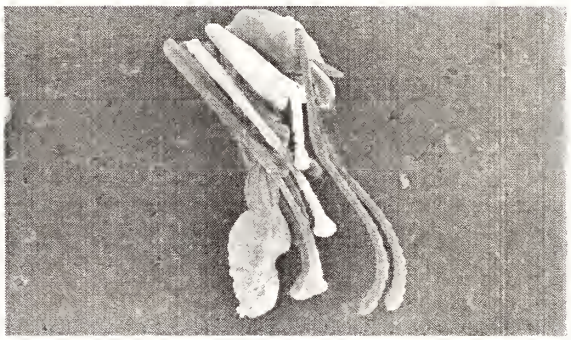

Fig.5

Fig.3g

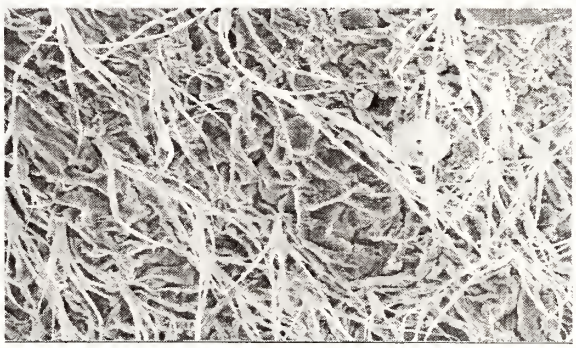

Fig.6a

Fig.3h

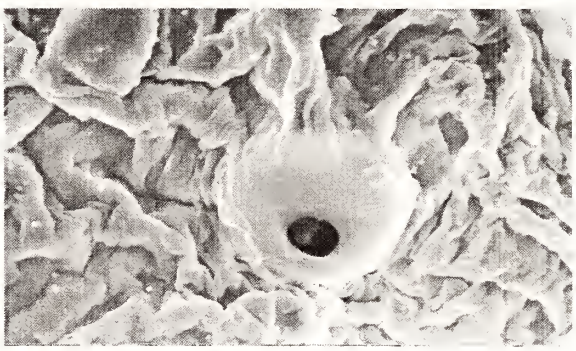

Fig.6b

different specimens within an instar has been observed. This variation was not reported earlier. Hairs are very sparsely distributed on the body. Length of the hair is very small. Longer and densely distributed hairs are present on the ventrally curved neck region of the thorax. There is no specific pattern of hairs on the head. Presence of cephalic seta on the tip of the cranium has been observed (Fig. 1h). Size of the seta decreases as the larva grows. Diana Wheeler (1982) considered the cephalic seta a feasible morphological character in establishment of number of instars. This character has been used during present study for confirming the number of instars for this species which were already established with the help of maximum head capsule width under optical microscope. Number of spiracles are 10 in all instars but diameter increases as the larva grows. Openings are unornamented in all spiracles in each instar. Width of the labrum increases in each instar (Fig. 1j, 2h, 2i, 3g and 3h). Arcila *et al.* (2002) described the larvae of *Paratrechina fulva* and observed considerable differentiation of mandible morphology between each larval instar. They considered mandible morphology a feasible character for identification of the larval instars. But in *O. smaragdina*, there is no noticeable difference between the mandibles in all instars. Length and width of the mandible increases (Fig. 2i, 2j, 3g and 3h). Presence of a prepupal stage in *O. smaragdina* has been indicated, which was not reported earlier (Fig. 4). Cuticular layer covers the larva completely and no internal structures were visible. Few head hairs in some specimens come out of the cuticle. Usually pupae of formicinae have a cocoon (e.g. *Camponotus*), but pupae in this species have no cocoon, like other exceptions in the subfamily (Wheeler and Wheeler, 1976) (Fig. 5). The female larvae (Fig. 6a and 6b) have a pattern of the body which is very different from all other larvae. Size of this larva is comparable to that of third instar larva. This larva is different from worker larva in terms of body hair coverage. As the worker larva has a very few, sparsely distributed hairs on its body, but the female larva is densely covered with bunches of multifid hairs.

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